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NUTRITION REVIEWS

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NEW YEAR'S GREETING TO THE READERS OF NUTRITION REVIEWS

The prompt reporting of a new research finding in a form that may be readily understood by those groups who can apply this finding for the benefit of the general public is of comparable importance to the increased support of fundamental research.

In the science of nutrition such groups include, in addition to the nutritional scientists, general medical practitioners, obstetricians, pediatricians, dietitians in hospitals and institutions, home economics teachers, public health workers, American Red Cross and welfare workers, science editors and writers, food editors, research directors and technical advisers in the food industry, and those who support fundamental research.

In its educational activities, the Nutrition Foundation deals chiefly with those groups that are in a position to pass along to the public, with confidence and least delay, new scientific information.

As one feature of this program, the Foundation has undertaken the publication of *Nutrition Reviews*, as an aid in bridging the gap between research findings and their application. This journal which was first published in November, 1942, provides an authoritative, unbiased, editorially interpreted review of the world's current research progress in the science of nutrition.

Its editorial staff is composed of young research personnel, selected chiefly from the biochemistry and medical fields. The editors are themselves active research workers in our strongest research and educational centers. They read regularly over two hundred scientific journals and periodicals, published here and abroad, for the material selected for review. An effort is made to maintain a high level of objective criticism of the world's current research literature in nutrition, providing an element of confidence and interpretation in regard to sound research findings and, conversely, a note of reservation when published reports are inconclusive and poorly interpreted. The assistant editors select and prepare the original reviews. Since their reviews are frequently modified by special referees and the editorial office, the editorial committee has felt that it was preferable not to affix the names of the editorial staff to individual reviews. Thus, there is a high degree of group responsibility on the part of the editor and assistant editors.

Nutrition Reviews has a most favorable response from subscribers in every state and territory of the United States, in all provinces of Canada, and in sixty (60) other countries. These latter countries include in part, Argentina, Australia, Belgium, Brazil, Bulgaria, China, Czechoslovakia, Denmark, Egypt, England, Finland, France, Germany, Greece, Holland, Iceland, India, Ireland, Italy, Mexico, New Zealand, Norway, Palestine, Poland, Rumania, Russia, Scotland, Siam, Spain, Sweden, Switzerland, Syria, Turkey, Union of South Africa, Wales, and Yugoslavia. Seven thousand five hundred copies are printed monthly and all but approximately five hundred copies are distributed. The circulation continues to grow and the cancellations are remarkably small.

A special feature of *Nutrition Reviews* is the index published in the last number of each volume. This is one of the most complete references on developments in the science of nutrition. With the completion of the fifth volume, it is planned to publish a cumulative five year index, which will provide an indispensable reference to all who are interested in the science of nutrition.

Dr. F. J. Stare, Editor, Dr. Elmer H. Stotz, Associate Editor, Mrs. Irene M. Kinsey, Assistant to the Editor, and all the Assistant Editors are to be congratulated for

a most useful and highly informative scientific publication.

A Spanish edition of *Nutrition Reviews*, "*Nutricion*," is distributed regularly from Mexico City for use in Latin American countries, through an arrangement with La Prensa Medica Mexicana. Two thousand copies of this publication are distributed monthly.

Recently while presenting an award of distinction to the Mayo Foundation for medical education and research, Dr. Isaiah Bowman, President of the Johns Hopkins University, said, "If I particularly mention the work of the Mayo Foundation in studies of the thyroid, it is only because detailed descriptions of the many Mayo Foundation achievements would hardly be suitable for an audience consisting so largely of laymen who may be somewhat appalled by scientific terminology, that 'iron curtain' which is customarily suspended between the investigator and the public."

While this statement was made partly in jest, it does raise questions as to whether scientific terminology serves as a barrier to laymen in being informed of the outstanding achievements of such institutions as the Mayo Foundation, and the research findings of scientists in universities and medical centers. Does this so-called iron curtain serve as a barrier to the general medical practitioner who has limited opportunity for reading to keep posted on the newer developments in the science of nutrition? Would the nutritional scientist receive greater and quicker recognition for his findings if his reports were prepared in a form that they could be more readily understood by the groups that deal directly with the public? Does it have an adverse effect upon laymen not professionally trained, who recognize the importance of fundamental research and generously contribute to its support? It would indeed be most unfortunate if scientific terminology should serve as a barrier to prompt application of research findings and to increased support of fundamental research.

In order to reach a large audience that is interested in nutrition, but is not trained in the sciences, The Nutrition Foundation since January 1, 1947 has been publishing monthly, "Current Research in the Science of Nutrition." The vocabulary of this leaflet based on current issues of *Nutrition Reviews* is adapted for lay reading and the subject matter is more restricted to clearly established findings of practical interest.

May I on behalf of the Nutrition Foundation say "thank you" to the many readers of *Nutrition Reviews* for their loyal interest. The Foundation is most anxious to expand and increase the usefulness of *Nutrition Reviews* in promptly passing along the advances in the science of nutrition in a form which may be readily understood by groups and individuals who are in a position to transmit such information to the public with a minimum delay. We welcome any suggestions from our readers.

Greetings for 1948!

OLE SALTHE
Executive Secretary
The Nutrition Foundation

AVAILABILITY OF RIBOFLAVIN AND THIAMINE FROM YEASTS

H. T. Parsons and J. Collord (*J. Am. Dietet. Assn.* **18,** *805 (1942)*) demonstrated that the riboflavin in living yeast cells was considerably less available for absorption by human subjects than the riboflavin in nonliving cells. Similar studies with bakers' yeasts which differed in viability and moisture content have been continued by E. L. Price, M. M. Marquette, and H. T. Parsons (*J. Nutrition* **34,** *311 (1947)*). Two general plans for metabolic studies were used: the first had been employed previously with alternate periods of three to five days for the feeding of the basal diet alone or with

supplements of a yeast or of crystalline riboflavin; the second had been devised by D. Melnick, M. Hochberg, and B. L. Oser (*Ibid.* **30,** *67 (1945)*) wherein a single test day was preceded and followed by periods of the basal diet. The subjects were university men and women students in a satisfactory nutritional state. The components of the basal diet were selected and prepared so as to guarantee a reasonably uniform intake of riboflavin throughout the entire regimen. Constant intake was sought through the use of unit lots of flour, canned peas, tomato juice, pineapple, and applesauce sufficient to last through the entire experiment. Supplies of meat loaf, orange-cranberry relish, and ice cream were prepared and frozen before the beginning of the investigation to be used throughout.

Fecal collections were marked by the ingestion of carmine at the beginning and end of each basal and supplementation period and were preserved in acid-alcohol. Collection of fecal material had to be omitted in those experiments where the one day test dose period was used. Duplicate riboflavin determinations on aliquots of the twenty-four hour urinary excretions and of the fecal collection were made on separate days by the fluorometric method of R. T. Conner and G. J. Straub (*Ind. Eng. Chem.* (*Anal. Ed.*) **13,** *385 (1941)*). Food aliquots representing 25 or 100 per cent of the weighed daily food intake were homogenized in a Waring blendor for riboflavin and thiamine assays. Analyses on aliquots of the composite diet indicated that the daily riboflavin and thiamine consumptions were 3.7 to 4.2 mg. and 1.2 to 1.5 mg., respectively.

In the first experiment, four samples of yeast were compared at levels of 45 g. per subject each day of the three to five day supplementation period, a level which contributed 825 micrograms of riboflavin per day. These samples had been prepared from one lot of yeast in the factory to rule out the variables which yeast strains and conditions of production might introduce. The first sample was fed as live, raw, fresh yeast, while the yeast cells in the second sample had been killed by boiling; the third sample consisted of cells dried under conditions which did not kill, while the fourth sample consisted of yeast dried under conditions which killed the cells. The urinary riboflavin excretion of the subjects which were fed the first sample containing live yeast cells did not increase above the basal excretion but the fecal riboflavin excretion was greatly increased. During the feeding of the third sample containing dried but live yeast cells, the urinary riboflavin level increased slightly and the fecal riboflavin increased greatly. In contrast, during the feeding of the second and fourth samples containing dead yeast cells, the urinary riboflavin excretion increased appreciably while the fecal riboflavin output remained constant.

Comparable investigations were made by the simplified, shorter procedure of Melnick, Hochberg, and Oser (*loc. cit.*). The percentage of a crystalline riboflavin supplement excreted by male and female subjects in these studies was in good agreement with data presented by Melnick *et al.* and averaged between 53 and 56 per cent for the various experimental groups. When the raw, fresh yeast used in the previous experiment was fed at a level of 150 g., i.e., 2.6 mg. of riboflavin, the average increase in urinary riboflavin excretion per day for the 5 subjects was only 12 mg. This increase represented an availability of only 9 per cent when compared with the increased secretion following a supplement of crystalline riboflavin. On the contrary the average increase in urinary riboflavin excretion after the ingestion of a specially dried nonviable yeast at a level which supplied 3.4 mg. riboflavin was 1.82 mg. When compared with the increased urinary riboflavin excretion after a test dose of 4.0 mg. of crystalline riboflavin, an increase of 1.82 mg. represented a complete availability of the ribo-

flavin in these dead cells. Other tests with commercial preparations of viable dried yeast cells indicated an availability of 11 to 18 per cent of the riboflavin in the yeast supplement in comparison with a 41 to 59 per cent excretion of the control test dose of riboflavin by the same subjects.

The data obtained by these two procedures demonstrate that the availability to human subjects of the riboflavin in yeast cells is inversely related to the viability of the yeast cells and not to their moisture content.

Human biologic assays have shown that bakers' compressed yeast when fed to human subjects is a poorly utilized source of thiamine as indicated by low urinary and high fecal thiamine eliminations (H. T. Parsons, A. Williamson, and M. L. Johnson, *J. Nutrition* **29,** *373 (1945)*; M. Hochberg, D. Melnick, and B. L. Oser, *Ibid.* **30,** *201 (1945)*). These data have been discussed in detail (*Nutrition Reviews* **4,** *45 (1946)*). Data in the former paper suggesting that possibly the ingestion of fresh yeast might be responsible for a reduction in the urinary thiamine excretion below that in basal periods, has been further explored by H. N. Kingsley and H. T. Parsons (*J. Nutrition* **34,** *321 (1947)*). The two types of bioassay technic employed in the above riboflavin experiments were used with the same basal diet for the thiamine investigations. Thiamine content of the various samples were determined by the thiochrome method of D. J. Hennessy (*Ind. Eng. Chem. (Anal. Ed.)* **13,** *216 (1941)*). When 15 or 150 g. of live yeast per day were fed for periods of five or ten days, the urinary thiamine excretion dropped below the basal level during the first day of yeast consumption and about the fourth day continued to decrease rapidly to an average daily level of 100 micrograms for the 15 g. supplement of yeast and 50 micrograms for the 150 g. supplement. A similar correlation was observed in the response to removal of live yeast from the diet: the longer the time on the large supplement of yeast, the slower the return to the previous output of urinary thiamine when the basal diet dose was again fed.

By the procedure of Melnick *et al.* (*loc. cit.*) the effects of three types of dried yeast were compared, in two of which the viability was retained and in the other destroyed. Drying the yeast had little or no relationship to the effect of yeast on the absorption of thiamine from the digestive tract but here again a definite correlation between viability of the yeast cells and thiamine availability was demonstrated.

The above data demonstrate the low availability to human subjects of the riboflavin and thiamine in live yeast cells. However, when the yeast cells were killed prior to ingestion, their riboflavin and thiamine were almost completely available. In thiamine metabolism during the ingestion of live yeast cells, not only was the thiamine in the yeast cells unavailable but the thiamine in the rest of the diet became less available as demonstrated by reduced urinary thiamine excretions. The data suggested that the live yeast cells in their growth and reproduction entered into active competition with the body for the thiamine in the digestive tract.

It would seem probable that other nutrients in the live yeast cells might likewise be less available to human subjects. These data indicate that the use of live yeast in any appreciable amounts as supplements to the human diet would not prove beneficial especially in terms of thiamine balance. However no evidence has been presented to indicate that nonviable yeast cells should not be recommended as supplements.

CYTOSIDEROSIS AND THE GENESIS OF ARTERIOSCLEROSIS

J. Gillman, T. Gillman, J. Mandelstam, and C. Gilbert (*Nature* **159,** *875 (1947)*) have put forth their ideas on cytosiderosis and the genesis of arteriosclerosis after

having contributed a good deal of preliminary work on the subject. Cytosiderosis is defined as "a diffuse metabolic disorder characterized by the deposition of large quantities of iron pigment, at first mainly in the liver and later in other organs and tissues of the body." This definition was established by earlier work (J. Gillman and T. Gillman, *Arch. Path.* **40,** *239* (*1945*); J. Gillman, J. Mandelstam, and T. Gillman, *South African J. Med. Sci.* **10,** *109* (*1945*)) in which it was shown that it occurs consistently in adult pellagrins, although it is commonly seen in other members of the African population "not suffering overtly from acute episodes of malnutrition."

By developing and applying a new technic of serial liver biopsies it has been shown that the iron pigment lodges first in the hepatic parenchyma and later in the reticuloendothelial system in the liver. Evidence from both biochemical and pathologic studies of the authors suggested that the second stage of the disease is characterized by a true siderosis by which the iron pigment is steadily disseminated via the gut mesenteric glands into the thoracic duct, and eventually by the blood circulation. They remark that the absorption of such large molecules "presupposes a disturbance in digestion and in the absorptive capacity of the gut epithelium."

To forward their discussion, the authors refer also to animal experiments, particularly the studies of J. Taylor, D. Stiven, and E. W. Reid (*J. Path. Bact.* **34,** *793* (*1931*); **41,** *397* (*1935*)). These workers showed that obstruction to the pancreatic ducts of cats fed a diet containing large quantities of iron results in the slow appearance of excessive amounts of iron in the liver, which was attributed to vitamin A deficiency impairing the efficiency of absorption by the gut epithelium. The authors' own experiments with cats have shown that when the pancreatic ducts are tied, iron can still appear in the liver when vitamin A is being actively absorbed, and that severance of the pancreatic duct and a high iron-containing diet "greatly expedite this extraordinary disturbance in iron metabolism in cats." Normal cats fed the same diet develop an "iron flush" in the Kupffer cells, while over the same period of time cats with obstructed pancreatic ducts accumulate massive quantities of iron pigment in the liver. From this the authors deduce that a badly balanced diet can lead to the accumulation of iron-containing molecules in the reticuloendothelial system.

These authors have also shown that the pigment in human cytosiderosis is a relatively inert molecule. The intravenous injection of this pigment into normal cats caused the accumulation of large masses of pigment, at first in the reticuloendothelial system in the form of coarse clumps and later in the liver cells situated near the medium sized portal tracts, such as they have described in the second stage of cytosiderosis in African pellagrins. The injection of colloidal iron in amounts equivalent to the iron present in the pigment and over the same period, leads only to a slight "flushing" of the Kupffer cells similar to that noted in intact cats fed the equivalent of approximately 40 mg. of iron daily.

The authors remark that it is of no immediate consequence whether the second stage of cytosiderosis in the African is associated with a disorder of the pancreas, but that the important issue for the purpose of the present communication, resides in the observation that sooner or later in cytosiderosis an inert molecule first found intracellularly in the liver is excreted via the bile, then absorbed by the gut and disseminated by the systemic circulation. The flooding of the circulation with macromolecules can result in a number of reactions which constitute the "macromolecular syndrome" as described by W. C. Hueper (*Arch. Path.* **33,** *267* (*1942*)) and others. Noteworthy features of this syndrome include the production of arteriosclerosis, fibrosis of the spleen, and curious hematologic reactions.

The authors point out that cytosiderosis

in the African occurs frequently in the third, and can be extensive in the fourth decade, which may mean that a large, inert, iron-containing molecule "is steadily flooding the circulation in minute amounts for ten or fifteen years" and that such a state of affairs could easily lead to those conditions optimum for the development of premature arteriosclerosis and other instabilities of the cardiovascular system known to occur in young Africans. They consider that for such reactions to occur, it would appear that chronic malnutrition associated with disease of the liver and the pancreas might also be essential attributes of the background of the African. They also consider that a new approach is now available for attacking a number of problems connected with the tendency to fibrous tissue reactions noted in many metabolic disorders and expressed as arteriosclerosis, hypertension, reticulosis, and cirrhosis.

AMINO ACIDS IN HUMAN URINE

It has long been recognized that free amino acids occur in human urine. Thus, H. G. Wells ("Chemical Pathology," *Ed. 5, pp. 627-632, W. B. Saunders, Philadelphia (1925)*) summarized many reports of the finding of leucine and tyrosine in the urine of patients with acute toxic necrosis of the liver. The occurrence of histidinuria in pregnancy (*Nutrition Reviews* **1,** *134 (1943)*) has been well established and in cystinuria, an inborn error of metabolism, quantities of free l-cystine are excreted. Other amino acids have been detected in the urine under various conditions. In addition to these free amino acids, biochemists have long been aware of the presence of several conjugated compounds which may occur in urine and which can be thought of as combined amino acids. Thus, hippuric acid contains glycine, mercapturic acids contain cystine, and the ill-defined group of "oxyproteic acids" probably contain several amino acids. In addition to free and combined amino acids, urine may contain many compounds which are derived from amino acids. Examples of these products are the keto derivatives, pyruvic acid, phenylpyruvic acid, and the like.

The development of microbiologic assays for amino acids (*Nutrition Reviews* **3,** *304 (1945)*; **5,** *56 (1947)*) has provided a convenient tool for studying urinary excretion of small amounts of amino acids. Some of the recent papers on this subject have been reviewed (*Ibid.* **5,** *43, 213 (1947)*).

W. Frankl and M. S. Dunn (*Arch. Biochem.* **13,** *93 (1947)*) have reported on studies of the apparent concentration of free cystine, tryptophane, and histidine in normal human urine. Urine was collected from 11 men and 9 women under various conditions. Some of the studies were made during periods of self-selected dietaries, others while the subjects were consuming army rations K or C. The microbiologic procedures used in the assays were methods previously reported. The "probable accuracy" of the assay data is stated by these workers to be as follows: tryptophane, range 1.5 to 10 per cent—most assays 2.0 to 3.0 per cent; histidine, range 1.4 to 8 per cent—most assays 2.0 to 3.0 per cent; cystine, range 3.4 to 10 per cent for most assays. The average urinary excretion of amino acids per twenty-four hours by women on self-selected diets was as follows: tryptophane 14.1 mg., histidine 117 mg., and cystine 100 mg. The excretion of tryptophane and histidine was somewhat greater by men, but about the same excretion by the two sexes was recorded for cystine. The values approximate those published by other workers who have employed microbiologic procedures. The rate of excretion of these three amino acids was maximum about three to four hours

following the ingestion of food. The average daily urinary excretion of these amino acids by male subjects maintained on the three dietary regimens was as follows: tryptophane, 20 mg. on C ration and self-selected diets, 40 mg. on K ration; histidine, 250 mg. on C ration, 200 mg. on K ration, and 175 mg. on usual diets; cystine, approximately 100 mg. on all three dietaries.

The values reported for the urinary excretion of tryptophane by Frankl and Dunn are considerably lower than those obtained by A. A. Albanese and J. E. Frankston (*J. Biol. Chem.* **157,** *59 (1945)*) when these latter workers used a colorimetric technic for the determination of tryptophane in urine. Albanese and Frankston found that healthy men (7 subjects) on self-selected dietaries excreted a mean of 281 ± 30 mg. of tryptophane daily. Frankl and Dunn corroborated the magnitude of this value obtained by the colorimetric procedure and further found that a sample of urine containing 270 mg. of "tryptophane" by the Albanese-Frankston method had no detectable quantity of tryptophane remaining after treatment with chloramine-T. These findings would be compatible with an interpretation that the microbiologic procedure measures free tryptophane while the colorimetric method estimates combined tryptophane as well. These "combined" forms of the amino acid appear to be susceptible to degradation with chloramine-T. The elucidation of the nature of this "combined" tryptophane will be awaited with considerable interest inasmuch as it may provide a clue to some peptide or conjugate of fundamental importance.

An accompanying paper from the California laboratory (W. Frankl, H. Martin, and M. S. Dunn, *Arch. Biochem.* **13,** *103 (1947)*) reports a survey of the excretion of apparent free tryptophane, histidine, and cystine in the urine of 57 patients with various disorders including liver disease, diabetes, malignancy, renal disease, blood dyscrasias, and pregnancy. No especial striking abnormalities were encountered, but almost one half of the values reported were either higher or lower than those encountered in healthy individuals. The excretion of histidine was most frequently different from that encountered in healthy persons. Urinary excretion of amino acids was high in some of the cases of pregnancy, liver disease, diabetes, and acute infection. Low amino acid excretion was observed in liver disease, renal disease, and malignancy.

Another paper from this laboratory (M. S. Dunn, M. N. Camien, S. Shankman, and H. Block, *Arch. Biochem.* **13,** *207 (1947)*) reports the twenty-four hour urinary excretion of nine of the amino acids essential for the rat (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine) plus glutamic acid, tyrosine, and glycine. The twenty-four hour urine specimens were preserved with toluene, stored in a refrigerator, and then hydrolyzed by heating for twenty hours on an oil bath with twelfth normal hydrochloric acid. The hydrochloric acid was removed and the individual amino acids determined microbiologically by methods which had been previously published. A test demonstrated that hydrolysis with urease did not alter appreciably the values obtained by microbiologic assay. The total twenty-four hour excretion of these twelve amino acids ranged from 1376 mg. to 2831 mg. for the men studied. Values for the women were somewhat lower. The excretion was nearly constant on a given dietary regimen. The lowest values reported are the amino acids arginine, isoleucine, leucine, methionine, valine, and phenylalanine. The highest excretion was of glutamic acid and glycine.

The data presented indicate that many of the amino acids are excreted in a conjugated or combined form inasmuch as the apparent concentration of the amino acids increased greatly after hydrolysis with hydrochloric acid. The most striking example of this is glutamic acid which could not be detected in

untreated urine, but was found in amounts of over 300 mg. per twenty-four hour sample after hydrolysis with hydrochloric acid. This is not too surprising when it is recalled that at least two peptides of glutamic acid are known to occur; namely, glutathione and phenylacetylglutamine (H. Thierfelder and C. P. Sherwin, *Ber. d. deutsch. chem. Gesellsch.* **47,** *2630* (*1914*)).

It appears probable that such studies as these will extend our present knowledge of the simple compounds of amino acids which are excreted in the urine and which may play important roles in metabolism. Interpretation of data obtained by microbiologic assay, however, must always be made in recognition of the incompleteness of knowledge regarding the response of some of the various test organisms to a great many compounds closely related to amino acids which occur in complex biologic fluids such as urine (*Nutrition Reviews* **3,** *304* (*1945*); **5,** *213* (*1947*)). The need is great for more extensive testing of the specificity of these various proposed microbiologic methods, especially as to keto acids, alpha-hydroxy acids, and the known conjugates which frequently occur in urine. As evidence for this need may be cited the fact that the apparent glycine concentration of urine was decreased by approximately 20 per cent by hydrolysis with hydrochloric acid. A footnote in the paper by Dunn *et al.* (*loc. cit.*) indicates that hippuric acid has a greater microbiologic activity per mole than has glycine for the test organism (L. mesenteroides P-60) employed in the assay.

DIABETES AND HEXOKINASE ACTIVITY

The report of W. H. Price, C. F. Cori, and S. P. Colowick (*J. Biol. Chem.* **160,** *633* (*1945*); see also *Nutrition Reviews* **4,** *78* (*1946*)) on the mechanism of insulin action seemed to bring together several isolated facts on the hormonal imbalance and etiology of diabetes. The investigations demonstrated that the enzyme hexokinase, which converts glucose to a phosphate form which can be utilized by other tissue enzymes, is inhibited both *in vitro* and *in vivo* by anterior pituitary extract, and that this inhibition is counteracted by insulin. In addition to the clinical implications of this report is the fact that this was the first demonstration of hormone action on a biochemical level. As such it was inevitable and essential that the original observations be confirmed in other laboratories.

R. H. Broh-Kahn and I. A. Mirsky (*Science* **106,** *148* (*1947*)) in performing experiments similar to those of the original investigators have been unable to subscribe to their conclusions. In the first place they prepared tissue extracts with hexokinase activity which although they were inhibited by some anterior pituitary extracts were not affected by others, though the latter were known to contain "diabetogenic activity." In the data presented, an illustrated anterior pituitary extract produced a 70 per cent decrease of hexokinase activity *in vitro*, and insulin completely restored hexokinase activity. On the other hand, a second pituitary extract produced no inhibition of hexokinase activity. Other than stating that the effect of anterior pituitary extracts was inconstant, no statement was given as to the number of experiments performed and hence the frequency of failure of the pituitary extracts. Elucidation of the effect of pituitary extracts will probably be clarified only with the availability of more purified "diabetogenic factor" from the anterior pituitary.

Broh-Kahn and Mirsky were careful to state that their anterior pituitary extracts were "quite different" from the extracts used by Price *et al.*, hence their results are not to be interpreted as lack of confirma-

tion of the previous results. The recent investigators have suggested that the active inhibitory factor of pituitary extracts may be labile or difficultly extracted, or may be some component of the extract other than the "diabetogenic factor." They also cast some doubt on the specificity of the pituitary factor by demonstrating that an extract of spleen may also produce some inhibition of hexokinase action. It may be noted, however, that the effect of splenic extract was small in contrast to that of the active pituitary extract. Since workers in the St. Louis laboratories (W. H. Price, M. W. Slein, S. P. Colowick, and G. T. Cori, *Federation Proceedings* **5,** *150 (1946)*) have found that adrenal extracts can also inhibit the hexokinase activity of muscle extracts, it is not improbable that inhibitory extracts may be prepared from other organs. It is perhaps significant that even in the case of splenic extract inhibition, insulin was able to counteract, even though the results were reported as inconstant.

By assaying muscle extracts from rats previously injected with anterior pituitary extract or made diabetic by alloxan, Price, Cori, and Colowick found the muscle hexokinase activity lowered as compared with that of normal animals. In contrast to these results, Broh-Kahn and Mirsky were unable to find a lowered hexokinase activity of muscle extracts from rats made diabetic with alloxan, nor could they find an effect of insulin on these extracts. Their general conclusion was that the diabetic disorders of glucose metabolism are not necessarily related to an inhibition of hexokinase activity.

It is difficult to resolve the differences in the results of these two groups of investigators since the differences are not simply those of interpretation, but of experimental results. Experimental differences may reside in the recognized variable character of anterior pituitary extracts, and possibly in the degree of the diabetic state produced by alloxan. Without more detailed data and study, it is difficult to evaluate possible differences in the experimental conditions.

The St. Louis investigators have been careful to point out that hexokinase inhibition and activation may only be a part of the picture of diabetes. Although the hexokinase mechanism is in accord with many of the facts of clinical diabetes, it is lacking in some respects. For example, in the hypophysectomized animal in which an inhibitory effect of the hormone should be absent, insulin nevertheless exerts its physiologic effect. According to the St. Louis studies, insulin only relieves the inhibition of hexokinase caused by the pituitary diabetogenic factor, but does not itself activate hexokinase. Yet it seems more than fortuitous that anterior pituitary extract and insulin exert the reciprocal effects on an enzyme system which is so essential to carbohydrate utilization. The inhibition and activation of enzymes may nevertheless be complex and indirect in nature, and where the observations bear such important metabolic implications as in the case of hexokinase, even more complete investigations are imperative.

SYNTHESIS OF VITAMIN A

Continuous advances have been made in the chemistry of vitamin A including two reported syntheses (*Nutrition Reviews* **4,** *267 (1946);* **5,** *64 (1947)*). It is not generally conceded that the past reported syntheses actually succeeded. There have now appeared two additional preliminary announcements of chemical synthesis of vitamin A.

D. A. van Dorp and J. F. Arens (*Nature* **160,** *189 (1947)*) who previously synthesized a "vitamin A acid," have now announced a vitamin A synthesis which uses the appro-

priate C_{18} ketone derivative of beta-ionone as the starting compound. This was treated with the ethoxy-acetylene described by T. L. Jacobs, R. Cramer, and J. E. Hanson (*J. Am. Chem. Soc.* **64,** *223 (1942)*) to yield an additional compound which was reduced by the action of suitable catalysts. By the action of hydrochloric acid and water, the ethoxy group was transformed to an aldehyde group. This vitamin A aldehyde was reduced by aluminum isopropoxide in isopropyl alcohol to yield an oily fraction which after chromatography appeared to contain about 35 per cent of vitamin A as determined by its ultraviolet absorption.

From the laboratories of Distillation Products, Inc. in Rochester, New York comes the preliminary report of J. D. Cawley and coworkers (*Abstracts, Am. Chem. Soc. Meeting, New York City, p. 26C, September (1947)*), who without presenting experimental detail claim the synthesis of pure, crystalline vitamin A and the crystalline acetate and anthraquinone carboxylate. It is reported that the biologic potencies and the physical and chemical properties of the natural and synthetic vitamins, as well as of their ester derivatives, are identical. The method of synthesis is not described, except to state that it is different from methods in the literature reported to produce compounds with vitamin A activity.

It would seem that detailed reports of either one or both of these two syntheses might conclude a difficult phase of vitamin chemistry. It would seem also that in the true spirit of scientific inquiry such detailed reports should be published rather than preliminary announcements.

NUTRITION AND NEUROLOGIC ABNORMALITIES IN MAN

Although neurologic abnormalities were among the first conditions in man to be attributed to nutritional deficiency, much confusion remains as to their classification and etiology. The observations made during and following the last war on internees and prisoners of war have added considerable information and have helped to clarify and classify the many diseases and syndromes described in the last one hundred or more years. D. Denny-Brown (*Medicine* **26,** *41 (1947)*) acting as Consultant in Neurology to India Command and to Allied Land Forces, South East Asia, made good use of the opportunity to observe the neurologic conditions, especially among prisoners of war. In addition he was able to collect data from other observers. These data, together with certain historical material, were classified and correlated in so far as possible with the dietary deficiencies and effects of therapy.

The author reports first on a group of 235 hospitalized prisoners following release from captivity in Burma. Nutritional deficiencies were not common in the group so that neurologic complications were few. However, 14 complained of loss of vision for reading and had central scotomata, 3 had ataxic gait, and 2 had the residual neuritis of beriberi.

From 3667 sick released prisoners of war from Singapore, Malaya, and Thailand the author studied all (380) who had neurologic disturbances. Of these, 110 had peripheral neuritis, 185 retrobulbar neuritis, 60 spinal ataxia, 13 deafness, 3 laryngeal palsy, and 9 spastic paraplegia. There were, in addition, 2 patients with pellagrous dermatitis and mental changes.

The peripheral neuritis was considered to be a manifestation of beriberi and was characterized by "the presence of symmetrical foot drop with muscular wasting below the knee, tenderness of calf muscles, stocking area of hypesthesia, and loss of the ankle jerks."

The retrobulbar neuritis began bilaterally and continued with progressive obscuring of the center of the field of vision. Complete

blindness was not observed. Recovery was slow in some instances, particularly the most severely affected. Central and, in some, paracentral scotomata were seen, and pallor on the temporal side of the disc was the principal fundal change regularly seen.

The degree of spinal ataxia varied greatly, but in most there was an unsteady gait, a wide base, and staggering on turning. The onset was insidious. Loss of vibration sense was regularly seen and in some extended to the clavicles and upper limbs. Position sense and touch and pain sensation were less often and less severely affected. Since a few of these patients had evidence of lateral column disease the similarity of this syndrome with subacute combined degeneration is discussed by Denny-Brown. However, as the author points out, pernicious anemia has been absent in all reported outbreaks. Furthermore, retrobulbar neuritis was frequently combined with the ataxic syndrome, but rarely complicates combined system disease.

The 9 patients with spastic paraplegia had a stiff legged gait with spontaneous ankle clonus and a tendency for the legs to cross in adduction. Knee and ankle jerks were hyperactive and led to clonus while plantar responses were extensor. No sensory abnormalities were observed in these patients, nor were mental abnormalities found.

Incomplete central deafness without vertigo was of relatively late and gradual onset and was seen in 8 of the patients with spinal ataxia and 5 with spastic paraplegia. Likewise in the 3 patients with laryngeal paralysis it was associated with ataxia and retrobulbar neuritis, except in one in whom the condition was thought to be true progressive muscular atrophy.

No exact count was made of the instances of the "burning feet" or "happy feet" syndrome, but not only had it been common in the camps but many residual instances were seen by the author. Peripheral neuritis was uncommonly observed with this syndrome and it was clearly thought not to be beriberi. This interesting syndrome has been known for many years and its history is reviewed by Denny-Brown.

M. Glusman (*Am. J. Med.* **3,** *211* (*1947*)) has added considerable first-hand knowledge of the "burning feet" syndrome as a member of a commission for its study at Bilibid Prisoner of War Hospital in Manila. "Burning in the soles of the feet" was described in 1826 in Burma and has since been observed not only in Bengal, Siam, Singapore, Malaya, but in many other parts of the world as well. It was seen in the Spanish Civil War of 1936 to 1939 and has been reported from a number of Japanese prison camps. Glusman has described the syndrome as gradual in onset with bilateral paresthesias in the feet which give way to a burning pain, worse at night, and relieved by cold. "Often when the pain was severe patients would sit cross-legged in bed, holding the distal portions of their feet in their hands and rock rhythmically backward and forward with the pain." Although sweating of the feet was sometimes seen during a paroxysm, color changes such as redness or cyanosis did not occur. The absence of these vascular changes serves to distinguish it from the acrocyanosis-like phenomena seen in some of the European prisoners (*Nutrition Reviews* **5,** *37* (*1947*)) and from such conditions as erythromelalgia.

There seems to be little doubt that the syndrome of "burning feet" is due to malnutrition. In fact, Glusman calls it "nutritional melalgia," and points to certain evidence that the syndrome may be due to a pantothenic acid deficiency. Although Glusman found thiamine and niacin ineffectual he was unable to try pantothenic acid. He does, however, discuss the suggestive work of C. Gopalan (*Ind. Med. Gaz.* **81,** *22* (*1946*)) who found dramatic improvement from the administration of calcium pantothenate. As Glusman points out, D. W. Woolley (*Proc. Soc. Exp. Biol. Med.* **46,** *565* (*1941*)) and R. H. Follis and M. M. Wintrobe (*J. Exp. Med.* **81,** *539* (*1945*)) have observed pain, suggesting painful limbs, in panto-

thenic acid deficiency in experimental animals. Further controlled observations on the effect of pantothenic acid in this syndrome in man are certainly called for.

In addition to a brief discussion of the "burning feet" syndrome, Denny-Brown has presented lucid reviews of the neurologic features of beriberi, pellagra, lathyrism, and a myasthenic bulbar paralysis.

With regard to beriberi the important point is made that unless strict adherence is held to the classic description of the disease with its cardiac abnormalities and specific peripheral neuritis, as quoted above, the term beriberi loses its meaning.

Lathyrism, a spastic syndrome, thought to be due to the inclusion of large amounts of the lathyrus pea in the diet, is discussed by Denny-Brown because of its possible relation to the spastic paraplegias he observed, although no final conclusion was drawn in this regard. Lathyrism has been known since early times (Hippocrates) and has been described in many parts of the world, especially in India. It is characterized by sudden onset with lumbar pain and weakness and stiffness of the lower limbs, rapidly progressing to a spastic paralysis. The lumbar pain and the paresthesias which also may accompany the onset of the syndrome soon disappear, but the spastic paralysis remains. Mental symptoms and sensory loss apparently do not occur. The diagnosis can only be readily made when spastic paraplegia occurs in epidemic form and is not related to other possible causes (ergotism, familial spastic paraplegia, and with the acute exanthemata). Although in most epidemics the diet contained a rather large concentration of lathyrus pea its relation to the disease is not completely clear. The author points out that the diets in all the epidemics so far as can be ascertained were low in protein, especially that derived from animal sources, and deficient in vitamins of the B-complex. Furthermore, the lathyrus pea is deficient in tryptophane and in this connection the author cites the recent evidence that tryptophane may at least in part replace dietary niacin and also points out the resemblance of lathyrism and the spastic syndromes observed to the neurologic conditions sometimes seen in pellagra. But, as he further points out, the relationship to pellagra is not a close one and, in fact, niacin did not appear to be beneficial. Thus, although lathyrism is associated with deficient food intake, especially of protein of high biologic activity, any etiologic relationship is not established, and, indeed, it is possible that a food contaminant may have the effect of a vitamin competitor or even a pharmacologic neuromotor effect.

Denny-Brown discusses a syndrome which he calls myasthenic bulbar paralysis reported from Singapore, although apparently he saw none among his cases. The condition is characterized by "tiring of the eyes" and blurring of vision followed by ptosis, diplopia, muscular weakness in the face and neck, dysarthria and dysphagia, and occasionally weakness of the arms and legs (*Nutrition Reviews* **4**, *208 (1946)*). No hint of the precise etiology of the condition is given, but presumably it is associated with chronic malnutrition.

Denny-Brown has clearly separated the following neurologic conditions presumably arising from faulty diet: the distal polyneuritis of beriberi, retrobulbar neuritis, spinal ataxia, spastic paraplegia, "burning feet" syndrome, deafness, and finally, the myasthenic bulbar syndrome. Except for the not unquestioned relationship of thiamine deficiency to the neuritis of beriberi, the etiology apart from nutritional deficiency common to all is unknown. The author concluded that the "burning feet" syndrome, retrobulbar neuritis, spinal ataxia, and spastic ataxia and paraplegia "represent isolated occurrence of the various neurological manifestations of pellagra." These or similar neurologic manifestations have certainly been repeatedly described with pellagra, but the small number

of typical pellagrous skin lesions and mental changes observed by the author would appear to stretch the definition of pellagra unduly. Furthermore, including them with pellagra does not necessarily aid in determining the ultimate cause in terms of specific nutrients.

As to therapy, crude yeast and liver preparations gave good results in some of the mild and early cases.

A POSSIBLE NEW REAGENT FOR THE DETERMINATION OF THE VITAMINS D

V. V. Palasi (*Nature* **160,** *88 (1947)*) reports a preliminary account of a new reagent for determination of the vitamins D. Physicochemical methods for the estimation of a vitamin in general depend upon either direct absorption spectrophotometry or colorimetry of reaction products. Since the maximum absorption of vitamin D_2 is at 265 millimicrons and has an E of 470 (1 per cent; 1 cm.), it is clear that there is little likelihood of using the first type of method, both because of the low absorption maximum and because it occurs at a wavelength where interfering substances are most troublesome.

Attention has therefore been directed chiefly to the possibilities of methods of the second type, and the most promising reagent hitherto used is a modified Carr-Price reagent, or solution of antimony trichloride in chloroform. C. H. Nield, W. C. Russell, and A. Zimmerli (*J. Biol. Chem.* **136,** *73 (1940)*) developed a fairly satisfactory reagent consisting of a solution of antimony trichloride and acetyl chloride in chloroform and determined the limits of concentration of these compounds. With vitamins D_2 and D_3 this reagent produces a yellowish pink color which reaches maximum intensity within thirty seconds and is stable for four to five minutes.

The spectral absorption curves of the colored compounds developed with vitamins D_2 and D_3 were shown to be identical, with a maximum at 500 millimicrons and an E value (1 per cent; 1 cm.) of approximately 1800. The optical density, determined by the difference in absorption at 500 and 550 millimicrons was directly proportional to the vitamin concentration, and the least quantity of vitamin D which could be accurately determined was 0.2 microgram.

This method was applied to the assay of fish liver oils by D. T. Ewing, G. V. Kingsley, R. A. Brown, and A. D. Emmett (*Ind. Eng. Chem. (Anal. Ed.)* **15,** *301 (1943)*) who used a chromatographic procedure for the removal of sterols and vitamin A. J. B. De Witt and M. X. Sullivan (*Ibid.* **18,** *117 (1946)*) reported the latest developments in the use of this reagent. They described a unique chromatographic technic, in that it employs a simple procedure permitting rapid separation of vitamin D from interfering sterols and vitamin A. Several commercial samples were assayed biologically and submitted to the new modification of the spectrophotometric test without the operators knowing that the bioassays had been performed. The figures obtained all appeared to indicate that the precision of the assay lay well within ± 2 per cent for high potency samples, although the authors did not feel that the question of the applicability of the procedure to low potency oils had been fully determined.

Palasi (*loc. cit.*) found a hitherto undescribed reaction product of the D vitamins, of "a clear and limpid blue color." He carried out Pettenkofer's reaction for the bile acids as follows: to 1 to 2 mg. of calciferol were added 1 to 2 mg. of sucrose and the mixture was dissolved completely in 1 to 2 ml. of absolute ethanol, and then 2 drops of concentrated sulfuric acid were added, giving a red color; then 6 drops of the acid were added, giving a stable blue color. After

shaking the test tube to mix the two layers, the color diffused throughout the liquid, and the final color is described as a "pale limpid blue."

The intensity of the color increased during the first fifteen minutes, after which it became stabilized as a "sky-blue" color. The author observes that the coloration obtained "seems to be in a direct relation to the concentration of the vitamin, according to Beer's law." In this preliminary report no quantitative spectrophotometric data or sensitivity measurements were made. The color produced by the provitamin ergosterol was different from that due to the vitamin itself, being a clear violet color.

INFLUENCE OF COBALT ON THE ANEMIA ASSOCIATED WITH INFLAMMATION

Polycythemia can be produced in many laboratory animals by administration of cobalt (M. O. Schultze, *Physiol. Rev.* **20,** *37 (1940)*; G. E. Cartwright, *Blood* **2,** *111, 256 (1947)*). Since the anemia associated with infection is refractory to therapy even though the bone marrow usually appears normal or even hyperplastic, M. M. Wintrobe and associates (*Ibid.* **2,** *323 (1947)*) studied the effect of this metal on the anemia produced by inflammation. The rat was used because polycythemia has been successfully produced in it by administration of cobalt. Four groups of rats from the Sprague-Dawley strain were used: one group was given cobalt; in another, inflammation was produced by injection of turpentine; a third group was given cobalt and injected with turpentine as well; a fourth group was used as controls. The rats were fed an adequate diet ad libitum.

As the animals matured the hemoglobin of the controls rose gradually. The hemoglobin of the rats injected with turpentine, on the other hand, failed to rise. The rats given cobalt showed a much more rapid rise in hemoglobin level than the controls and these animals ultimately attained polycythemic levels. Of special interest was the fact that the rats injected with turpentine but given cobalt as well developed nearly the same degree of polycythemia as the animals given cobalt alone. Weight gain tended to be impaired both by the injection of turpentine and by administration of cobalt but was influenced more consistently by the latter.

These experiments were carried out three times, using a total of 108 rats, and the results were the same each time. The impairment of hemoglobin production which resulted from the injection of turpentine was associated with a reduction in the plasma iron level and an increase in erythrocyte protoporphyrin just as had been observed in patients with anemia accompanying infection (G. E. Cartwright *et al.*, *J. Clin. Invest.* **25,** *65 (1946)*). The simultaneous administration of cobalt and turpentine did not seem to make a significant difference in the degree of hypoferremia associated with the administration of turpentine even though the impairment in hemoglobin production was largely overcome. The degree of increase in erythrocyte protoporphyrin which was observed, appeared on the whole, to be related inversely to the degree of hypoferremia. Administration of cobalt alone tended also to be associated with the development of lower levels of plasma iron as compared with those in control animals and there was at the same time an increase in erythrocyte protoporphyrin. Somewhat higher levels of serum copper were noted in association with the administration of cobalt and the injection of turpentine than were found in the control animals but it could not be determined whether the differences observed were truly significant. Total plasma protein levels were also measured. The protein level was observed to be reduced in all the groups as compared with the controls, the reduction apparently being due to a reduction in the

plasma albumin fraction. Nitrogen excretion, however, as measured by urinary urea plus ammonia was the same in all groups of rats.

It would thus appear that the anemia induced by inflammation could be in large measure overcome in the rat by the administration of cobalt but this was accomplished without modifying significantly the other changes which have been noted in association with inflammation; namely, loss of weight, reduction in plasma albumin, hypoferremia, increase in erythrocyte protoporphyrin, and increase in plasma copper.

There is no clue as to how cobalt produces this effect. As yet, the mode of action of cobalt in producing polycythemia in normal animals is not known. In this respect, the observation of K. Kato and V. Iob (*Am. J. Clin. Path.* **10,** *751 (1940)*) that the spleen and bone marrow of dogs and rabbits fed cobalt in addition to iron contained less iron than those of animals given iron alone, is of special interest. This observation would suggest a more complete utilization of iron for erythropoiesis in the presence of cobalt. Since a profound disturbance in iron metabolism occurs in the presence of inflammation, one wonders whether cobalt in some way influences and improves the utilization of iron, counteracting, to some extent, the influence of inflammation. Studies by G. R. Greenberg, H. Ashenbrucker, M. Lauritsen, and M. M. Wintrobe (*J. Clin. Invest.* **26,** *114 (1947)*) have shown that the hypoferremia associated with infection is not due to a lack of iron and cannot be corrected by infusing iron intravenously but must be assumed to be due to some disturbance related to iron metabolism whereby utilization is affected.

METHIONINE AND CYSTINE CONTENT OF THE HEN'S EGG

F. A. Csonka, C. A. Denton, and S. J. Ringel (*J. Biol. Chem.* **169,** *259 (1947)*) have investigated the content of methionine and cystine of eggs as influenced by the diet of the laying hen. Changes caused in the composition of the egg, especially in distribution of the indispensable amino acids, would be regarded as significant findings because of their effects upon the biologic value of the whole protein. The study is of particular interest to human nutrition since eggs are considered to be one of the most valuable supplementary foods.

Available data from earlier work do not give clearcut answers to the questions which arise. W. D. McFarlane, H. L. Fulmer, and T. H. Jukes (*Biochem. J.* **24,** *1611 (1930)*) observed no change in the amino acid composition of the egg proteins when the basal diet of the hens was varied from 10 to 16 per cent protein level, although it now appears that a higher level of protein would have been more appropriate in testing this dietary influence for its effect on the amino acid composition of the egg protein.

No change in the amino acid distribution in egg albumin and in vitellin separated from eggs laid by hens fed a diet in which the protein was supplied entirely by wheat, corn, or soybeans respectively, was observed by H. O. Calvery and H. W. Titus (*J. Biol. Chem.* **105,** *683 (1934)*). On the other hand, working with pigeons, L. Gerber and R. H. Carr (*J. Nutrition* **3,** *245 (1930)*) by means of similar experiments, observed differences in the nitrogen distribution of the egg proteins and in the immunologic behavior of rats sensitized to egg protein, pointing to the probability of change in the amino acid composition of the egg protein induced by ingestion of the various feeds.

If the amino acid composition of the different egg proteins is considered constant, then a change in the amount of any given amino acid in the egg may mean that the total protein of the egg has been changed or that the relative proportions of the different

egg proteins has been altered. All of the amino acids would naturally increase together with an increase of total protein content, so that the ratio of any two amino acids, such as cystine and methionine would not change.

Rhode Island Red hens in their second laying year were used for the experiments. The hens were kept in laying batteries, on a low protein diet for a period of a month. The diet contained ground yellow corn 87, alfalfa leaf meal 5, steamed bone meal 4.5, butyl fermentation solubles 0.5, limestone flour 2, manganized salt 0.5, iodized salt 0.2, vitamins A and D oil 0.3, and corn starch 6.0 parts. The fermentation solubles provided sufficient riboflavin, and the diet as a whole provided 9 per cent protein, with 100 g. containing 0.18 g. cystine and 0.26 g. methionine.

Three high protein diets were prepared from this by replacing 20 parts of corn meal by casein or gelatin, or by replacing 50 parts of corn meal by soybean meal. Of these three diets, the casein high protein diet contained the most methionine (0.75 per cent) although it contained only 0.22 per cent of cystine. The soybean-containing diet had 0.4 per cent of methionine and of cystine, while the gelatin high protein diet contained 0.36 per cent of methionine but only 0.16 per cent of cystine. The methods of egg collection and analysis were those previously described by F. A. Csonka, H. Lichtenstein, and C. A. Denton (*J. Biol. Chem.* **156,** *571 (1944)*) and by F. A. Csonka and C. A. Denton (*Ibid.* **163,** *329 (1946)*).

The three proteins used in raising the protein content of the low protein diet were different as to origin as well as in methionine and cystine content. Changes occurred in the weight of egg from a given hen, and in the cystine, methionine, and nitrogen content of an egg. These changes occurred both on the low and on the high protein diet. There was no definite consistent relationship between the weight of the egg and its cystine, methionine, or nitrogen content, although in general the larger eggs did contain the most nitrogen. The fluctuations which occurred in the methionine and cystine content of the eggs on all types of diets examined did not necessarily bear any relationship to each other. Presumably these fluctuations are normal physiologic variations which may be caused by factors limiting the rate of supply of the necessary components for synthesis of the proteins of the yolk and the white. Theoretically the hen could supply more methionine from its own reserve protein to complete the synthesis of egg protein; this does not seem to happen. When the dietary protein is 20 per cent gelatin and therefore incomplete, egg production ceases, so that the hen relies on the feed for the synthesis of egg protein.

With the high protein diet containing 20 per cent casein the average cystine and methionine content of the eggs, as well as their weights, were higher than those laid while receiving the low protein diet; and the differences were statistically significant. The high protein diets containing either soybean meal or gelatin yielded eggs which tended to be lighter and to contain less methionine than the eggs laid while the low protein diet was being fed. The high casein diet caused eggs to be laid with higher cystine content than did the soybean meal diet, although the latter diet contained twice as much cystine as the former.

The authors suggest that this result is most plausible on the hypothesis that the cystine is synthesized from other components of the diet, particularly methionine. The supplementation of the diets described with synthetic methionine did not materially raise the methionine or cystine content of the eggs; indeed when more than 0.5 g. of methionine was given daily, it appeared to exert an effect in stopping egg production. The authors felt that only when methionine was present in the diet in peptide form did an increase in its quantity lead to the appearance of more of the amino acids in the egg.

They also believed that the amount of these amino acids in the egg cannot be considered to be constant but may depend on the hen's dietary protein. The experiments described reveal the importance of both the quantity and quality of the feed protein in establishing high levels of the sulfur-containing amino acids in the egg.

SOME HOMOLOGOUS ANTIBIOTIN COMPOUNDS

K. Dittmer, M. F. Ferger, and V. du Vigneaud (*J. Biol. Chem.* **164,** *19* (*1946*)) described the synthesis of four homologous imidazolidone aliphatic acids in which the imidazole groups were substituted in valeric, caproic, heptanoic, and octanoic acids respectively. K. Dittmer and V. du Vigneaud (*Science* **100,** *129* (*1944*)) and R. Duschinsky and L. A. Dolan (*J. Am. Chem. Soc.* **68,** *2350* (*1946*)) had reported that dl-imidazolidonecaproic is an antibiotin for Lactobacillus casei and for Saccharomyces cerivisiae. K. Dittmer and V. du Vigneaud (*J. Biol. Chem.* **169,** *63* (*1947*)) now report the relative antibiotin activities of all four imidazolidone aliphatic acids for these two organisms and describe in detail the microbiologic methods used for the determination of these values, and discuss the avidin-combining abilities of these compounds.

Antibiotin is expressed as the molar inhibition ratio, which is defined as the number of molecules of an antibiotin required to inhibit 1 molecule of biotin. It is determined experimentally by finding the concentration of an antibiotin which is able to reduce the growth obtained with 0.0002 microgram of biotin to that obtained with 0.0001 microgram of biotin. The molar inhibition ratio can be calculated after the conversion of these amounts to molar quantities, and of course the smaller the inhibition ratio, the greater the antibiotin activity of a compound.

The experimental data for determination of the inhibition ratios of three of the imidazolidone aliphatic acids are presented in a graph giving curves for each; with S. cerivisiae the growth level with 0.0002 microgram of biotin per 7 ml. was reduced to that with 0.0001 microgram by just over 200 micrograms imidazolidoneoctanoic acid, or by approximately 175 micrograms imidazolidoneheptanoic acid, or by somewhat less than 100 micrograms imidazolidonecaproic acid. It is thus evident that imidazolidonecaproic acid, which has an aliphatic acid residue of the same chain length as that found in desthiobiotin, is a more powerful antibiotin than its next two higher homologues. The effect of biotin in nullifying the inhibitory action of the imidazolidone aliphatic acids on the growth of the yeast was also determined, with the growth tube containing 400 micrograms of the inhibitor in 7 ml. Approximately 5, 6, and 10^{-4} micrograms of biotin in the 7 ml. of medium were required to give a 50 per cent reversal of the inhibition due to imidazolidoneoctanoic, imidazolidoneheptanoic, and imidazolidonecaproic acids respectively.

The action of the imidazolidone aliphatic acids was also tested against the growth of L. casei under standard conditions, the molar inhibition ratio was 52×10^6, 10^5, 1.6×10^6, and 10^7 for imidazolidonevaleric, -caproic, -heptanoic, and -octanoic acids respectively. The inhibition ratios of these four acids against desthiobiotin also were arranged in the same order, and for the valeric acid (the least inhibitory) 0.034 microgram of desthiobiotin was required to counteract halfway the toxicity of 500 micrograms.

In each type of activity demonstrated for the imidazolidone aliphatic acids, the caproic acid is the most active. This is the homologue which has an acid residue with the same carbon chain length as that found in desthiobiotin, from which it differs only in the absence of a methyl group attached to

a different position of the ring nucleus. Shortening of the aliphatic acid carbon side-chain to valeric acid markedly reduced the antibiotin activity of the imidazolidone aliphatic acid; lengthening of the acid side-chain to heptanoic and octanoic acid progressively diminished the antibiotin activity, but to a lesser extent than shortening the chain by 1 carbon atom.

Since the substituted aliphatic acids examined contain the same cyclic urea group as biotin itself, and since this group has been regarded as essential to the avidin-combining power of biotin (V. du Vigneaud, K. Dittmer, K. Hofmann, and D. B. Melville, *Proc. Soc. Exp. Biol. Med.* **50,** *374 (1942)*), the authors made preliminary measures of the power of each of the imidazolidone aliphatic acids to combine with avidin, and have shown that the avidin-combining powers of the acids vary in the same manner as their antibiotin activities.

The imidazolidonecaproic acid also inhibited the growth of types II and III pneumococci and Escherichia coli, although it must be remembered that antibiotin activity was in all cases counteracted by the addition of biotin. The imidazolidone aliphatic acids were much more potent inhibitors of yeast growth when desthiobiotin was the growth stimulant than when biotin was used.

NUTRIENTS IN MILK FROM ANIMALS OF DIFFERENT SPECIES

Because of the importance of milk for the nutrition of young mammals, many studies have been made of the various nutrients contained therein. Principal attention has been paid to cow's milk, due to its commercial importance, and somewhat less emphasis has been given to human milk and goat milk (*Nutrition Reviews* **3,** *118, 203 (1945);* **4,** *134, 211 (1946)*).

The milk of other species has been studied in a few laboratories; vitamin contents, fat, sugar, total solids, and other values have been determined, so that comparative values are now available. One of the early vitamin studies was made by J. Houston and S. K. Kon (*Biochem. J.* **33,** *1655 (1939)*) who determined the thiamine and riboflavin content of rat and guinea pig milk. Later, R. J. Williams, V. H. Cheldelin, and H. K. Mitchell (*Univ. Texas Pub.* **4237,** *97 (1942)*) recorded the levels of eight B-vitamins in the milk of six species, including eight samples of human, one mare, twenty-eight cow, four goat, one dog, and two of mouse milk. Microbiologic assay methods were used throughout by the latter group, and in the light of more recent experience it seems possible that the enzyme-digested samples which these workers used may have yielded low results, particularly with respect to biotin and pyridoxine. On the other hand, relative values obtained among the different species provide comparative data. Special ultramicromethods were employed for the determination of mouse milk, since the total volume of the samples varied from 0.04 to 0.08 ml.

When the different species were arranged in the order of decreasing time to reach maturity (which furnishes an order of increasing protein content of the milk solids) the B-vitamin levels tended to increase as well. The levels in the mouse were consistently highest and the dog second, except for thiamine. Mouse milk was usually ten to fifty times as rich in the various vitamins as human and mare milk, which were generally the poorest sources. The guinea pig and rat values obtained by Houston and Kon were in agreement with the trends outlined by the Texas workers.

Some notable relationships were observed with respect to niacin, riboflavin, and folic acid. Niacin, which is low in cow's milk, usually averaging less than 1 microgram per milliliter, was present at about twice this

level in human milk. Riboflavin, on the other hand, was low (0.4 microgram per milliliter) in human milk, and mare's milk contained only 0.02 microgram per milliliter. (Cow's milk generally contains 1 to 3 micrograms of riboflavin per milliliter.) Folic acid was relatively high in the human and dog milk (approximately 0.13 microgram per milliliter), whereas the milk of the herbivorous animals contained less than 0.02 microgram per milliliter. Finally, it was pointed out that from the standpoint of vitamin nutrition, goat milk was significantly superior to cow milk in niacin only.

The vitamin content of mare's milk has been determined in 3 animals by A. D. Holmes and co-workers (*J. Dairy Sci.* **29,** *163 (1946)*) who confirmed the low riboflavin values above, but found niacin, thiamine, and ascorbic acid levels which were comparable with those in cow's milk. The lactose content of mare's milk is slightly over 6 per cent (R. G. Linton, *J. Agr. Sci.* **21,** *669 (1931)*) a figure which is nearly as high as in human milk (approximately 7 per cent) and is considerably higher than the level in cow's or goat's milk. Holmes has pointed out that mare's milk is probably more nearly like human milk than that of any other animal, and in a subsequent publication A. D. Holmes, A. F. Spelman, C. T. Smith, and J. W. Kuzmeski (*J. Dairy Sci.* **30,** *385 (1947)*) compared mare's milk with that of several other species, with respect to water, protein, ascorbic acid, ash, magnesium, potassium, calcium, and phosphorus contents. The data compiled include their own assays of twenty-eight samples of milk from 4 mares, as well as a summary of the values obtained by other workers for human, mare, cow, goat, ewe, buffalo, camel, sow, and elephant milks. Mare's milk is low in fat (1.0 to 1.6 per cent) and this accounts for virtually all of the discrepancy in solids content that exists between mare's milk and human milk. The approximate values for human, mare, and cow milk were as follows: water, 87.5, 89.7, and 87 per cent; protein, 1 to 2, 2.3, and 3.3 per cent; reduced ascorbic acid, 50, 80, and 12 to 25 mg. per liter; ash, 0.2, 0.4, and 0.7 per cent; phosphorus, 15, 60, and 80 mg. per 100 g.; calcium, 30, 100, and 120 mg. per 100 g. Potassium and magnesium values for human milk were not given.

A somewhat undesirable feature of the data presented in the above summary is the rather wide variability among assay values. This is usually most pronounced among vitamin values. Some of the variations have probably been due to differences in feeding and environmental factors, as well as in the methods of assay. In an effort to minimize the differences in vitamin values in cow's milk, J. J. Stefaniak and W. H. Peterson (*J. Dairy Sci.* **29,** *783 (1946)*) assayed representative samples of commercial milk obtained from three companies whose combined output was over 100,000 pounds daily. Twenty-nine samples were obtained at five dates in the fall, winter, and spring. Thirty-six samples from experimental farms, taken over a two year period, were included for comparison. Values were reported for pantothenic acid, niacin, and biotin. The extreme fluctuations which have characterized some earlier reports were eliminated, although variations were exhibited among the commercial samples. Pantothenic acid values ranged from 2.7 to 4.5 micrograms per milliliter, with only one sample containing more than 3.9 micrograms per milliliter. Niacin values ranged from 0.63 to 1.08 micrograms per milliliter, with an average figure of 0.80 microgram per milliliter. Biotin values varied from 0.017 to 0.038 microgram per milliliter, with an average of 0.029. The niacin content of the experimental milks increased slightly during the summer months, but the biotin values showed a marked decline during June when the cows were pasture fed. The biotin values fluctuated considerably among the experimental cows (0.020 to 0.059 microgram per milli-

liter) whereas the niacin values varied only about 10 per cent and the pantothenic acid values 20 per cent in these animals. Since biotin is elaborated in large amounts by the bacterial flora, it was considered likely that changes in pasture conditions and silage feeding might easily bring about wide variations in biotin production. The relative constancy of values for the other two vitamins, on the other hand, suggests that feeding factors are less important here, and that vitamin values for milk can be expressed with fair precision if the assay routine and other experimental procedures are properly controlled.

Studies such as the ones outlined are helpful in obtaining a better understanding of comparative nutrition of animal species. The B-vitamins in particular seem to parallel roughly the protein content of the milk, which in turn is in accord with the metabolic rate of the animal, and in inverse ratio to the relative growth. Although the principal use of mare's milk in this country is for raising foals, it has been used for human food in sections of Western Asia.

USE OF A MIXTURE OF PURE AMINO ACIDS IN SURGICAL NUTRITION

Studies on the use of protein hydrolysates for nutritional purposes (*Nutrition Reviews* **5,** *329* (*1947*)) have revealed that they present certain limitations (R. Elman, *Med. Clin. North America* **27,** *303* (*1943*)). Thus, the rate of intravenous administration is limited and the volumes required are large. To administer the equivalent of 90 g. of protein takes eight or nine hours and a volume of 2500 ml. Febrile reactions, nausea, and vomiting may occur, and the material may be irritating subcutaneously.

S. C. Madden, F. W. Anderson, J. C. Donovan, and G. H. Whipple (*J. Exp. Med.* **82,** *77* (*1945*)), after testing various mixtures of pure amino acids in respect to their efficiency in regenerating plasma protein in the dog subjected to plasmapheresis, came to the conclusion that one mixture (VUJ) was useful. An extensive study of the value of this amino acid mixture has been undertaken by S. C. Werner (*Ann. Surgery* **126,** *169* (*1947*)).

The material used was a mixture of the ten essential amino acids and glycine. It was given as an 8 per cent solution which forms a clear, stable, bitter tasting fluid, light greenish yellow in color. The solution has a pH of 5.5. Because of this acidity, 480 ml. of one-sixth molar sodium lactate solution was given to provide base when large amounts of the amino acids were used parenterally. It was found best to give these solutions separately rather than mixed. Of the amino acids, 14 per cent are dextrorotatory and presumably unavailable for protein anabolism.

An infusion of 1500 ml. of this solution provides the average daily requirement of 15 g. of available nitrogen, about 95 g. of protein equivalent. This can be given in four hours. About 350 calories are furnished in this way since most of the dextrorotatory and presumably physiologically inactive isomers are also burned. From this must be deducted 1 to 4 g. of amino acid nitrogen which are excreted unchanged in the urine and so are lost for combustion purposes—about 80 calories.

The study was carried out in a total of 73 cases, some for periods up to three weeks and in amounts up to 3 liters per day. A total of about three hundred and fifty intravenous infusions were given. The material was also given by hypodermoclysis in about twenty-five instances. The infusions generally were the sole source of nitrogen. They were then supplemented with 1500 to 3000 ml. of a 5 per cent dextrose-saline solution. In other instances, food was given, the amino acid solution being pro-

vided to supplement an inadequate protein intake.

Nausea and vomiting were the only frequent ill effects of amino acid therapy and these appeared to be related to the rate of administration and amount given. Amounts up to 1 liter per hour were given without reaction but in general 400 ml. per hour was the maximum speed consistently tolerated. Although few determinations of blood amino nitrogen were made, in so far as the studies went, nausea, in general, was found consistently with levels over 20 mg. per cent. Hyperpyrexia and chills occurred in 5 patients but these may have been the result of faulty care of the infusion sets. Cellulitis was noted only once in the twenty-five instances in which amino acids were given subcutaneously. The carbon dioxide content of the blood was reduced by the administration of more than 2000 ml. per day of the amino acid solution and the fall was not prevented by the concurrent administration of 12 g. of sodium chloride as a 5 per cent dextrose-saline solution or by the use of the molar lactate solution already mentioned. One death occurred and this was associated with a drastic reduction in blood carbon dioxide content (16 volumes per cent).

It was found feasible to give as much as 1500 ml. of the amino acid solution twice daily. In such cases the quantity of dextrose-saline solution was reduced to 1500 ml. so as not to overload the circulation. At a rate of 400 ml. per hour even patients known to be on the border of cardiac failure tolerated large amounts of amino acid without difficulty. In such patients, however, the glucose supplement was given by clysis. That the great bulk of the amino acid was converted into urea was evidenced by the rise in urea nitrogen in the blood and by the greatly increased urinary nitrogen. The amino acids were not used where there was any indication of renal damage in view of the acidosis and the rapidity of urea nitrogen accumulation in the blood which resulted with even normal kidney function. The impression was gained that emaciated patients who could not afford to lose from their fat stores to build up their body protein stores could only be treated for short periods by this technic unless some calories were taken by mouth. However, the still relatively well nourished hypoproteinemic patients could build up protein in this way at the expense of fat for long periods even when the amino acids were used to supplement an inadequate food intake.

Particular attention was given in this study to the nitrogen balance since it is well established that one of the results of injury to the body is the appearance of a negative nitrogen balance on the same nitrogen intake which previously maintained equilibrium. The balance studies were carried out in 2 young, healthy orderlies, in 15 patients preoperatively, and in 29 patients after operation. Twenty-one of the last underwent partial gastrectomy for peptic ulcer while the remainder had a variety of operative procedures. Six additional gastrectomy patients were followed as controls in that they were treated in the exact way as other gastric cases but were given no amino acids. The general procedure was to reduce the amount of amino acid infusion as the protein intake by mouth increased. Thus, most patients received amino acids in decreasing quantities for about six days after operation.

The least amount of nitrogen lost by any of the 6 control patients subjected to partial gastrectomy and given no amino acid postoperatively was 25 g. of nitrogen in the first five days after the procedure. Seventeen of the 21 patients undergoing the same procedure but treated with amino acids lost varying amounts less than this. Three of these 17 patients remained in nitrogen balance for this period and 6 showed actual positive balance. That there was considerable wastage of amino acid material nevertheless was shown by the high urinary nitrogen excretion. Some of this wastage, it is suggested, may have been the result of the low amount of calories supplied, forcing

thereby a diversion of part of the amino acids for fuel. An interesting observation was made in 3 patients. When amino acid administration was stopped before an equivalent intake was achieved in the diet, the excretion of urinary nitrogen continued at a high level. This produced a sharp negative nitrogen balance which was sufficient in a day or two to exceed the total negative balance of the controls for the entire first five days after operation. By reducing the amino acid output only as food intake was increased to the same degree, such a negative "shift" could be prevented.

Inanition and debility abolished the tendency toward negative nitrogen balance which usually occurs after injury. Although there was considerable variation, a low nitrogen intake of 0.11 to 0.30 g. of nitrogen per kilogram of body weight was generally associated with negative balances and higher intakes were accompanied by nitrogen-sparing, if not balance. The total sulfate excretion in the urine tended to parallel nitrogen output, the sulfur:nitrogen ratio averaging about 1:15. Thus the variations in nitrogen balance probably involved tissue protein sources.

Of 8 operative patients other than the gastrectomy cases, nitrogen loss was decreased in 4, and 2 fracture patients treated in this way were maintained in nitrogen equilibrium.

The clinical study of these patients indicated that a fifth of the controls appeared to do less well than the amino acid treated cases. Eleven of the control cases stayed in the hospital considerably longer than any of the treated cases, their temperatures failed to return to normal as quickly, and they did not eat as well. Furthermore, 5 of these developed objective difficulties at the gastroenterostomy stoma with gastric retention whereas none of the amino acid treated cases showed this complication. However, the results in the two groups were similar enough to raise the suspicion that routine, unconsidered, postoperative therapy with hydrolysates or amino acids may be in large part unnecessary.

The loss of nitrogen observed following injury has been referred to as the toxic destruction of protein and received attention as long ago as 1894 (R. May, *Z. f. Biol.* **30,** *1 (1894)*). The studies of D. P. Cuthbertson (*Quart. J. Med.* **1,** *233 (1932)*) revived the concept that injury is followed by a phase of catabolism. This conclusion was reached because Cuthbertson could not restore his patients to nitrogen balance even with moderate increases in nitrogen intake. Werner points out that a true catabolic response implies that stopping nitrogen intake from the time of injury would, if anything, increase the loss of nitrogen from the body. The data of his study contradict this. No excesses of nitrogen appeared in the urine when nitrogen was withheld postoperatively and, in fact, the nitrogen excretion was sometimes less than that of an uninjured patient on the same intake.

A different theory to explain the inability to spare nitrogen postulates that incoming nitrogen is routed through the liver after trauma and is wasted before it can be used for anabolism by the tissues. The loss of nitrogen is "antianabolic" rather than "catabolic" (C. M. Grossman *et al.*, *J. Clin. Invest.* **24,** *523 (1945)*). However, the data of Werner's study indicate that incoming nitrogen is not entirely wasted and show that a high nitrogen intake lessens or reverses negative nitrogen balance after trauma. The observations of other workers such as Co Tui *et al.* (*Ann. Surgery* **120,** *99 (1944)*) are similar to those of Werner.

Unable to accept these theories, therefore, Werner suggests that there is an increase in the rate of both anabolism and catabolism in association with injury rather than a change in either. This suggestion would explain the fact that an increased nitrogen intake can reestablish nitrogen equilibrium and that more or less than this amount results in varying degree in positive or negative nitrogen balance as in the healthy subject.

This also explains the continued outpouring of nitrogen from the tissues when the high nitrogen intake is suddenly stopped. The fact that such evidence of increased metabolism is not present if nitrogen has not been given after the trauma indicates one necessary condition in addition to the injury for the response to be elicited, namely, the presence of incoming nitrogen. Another condition is that there should not have been recent preceding injuries or body wasting from inanition. S. C. Madden and W. A. Clay (*J. Exp. Med.* **82,** *65 (1945)*) have pointed out that reserve protein stores are necessary for the reaction to trauma to occur. A third factor is the severity of the injury in that the more severe the insult the more nitrogen is needed to restore equilibrium.

Since in some patients restoration to normal nitrogen balance or sparing of nitrogen was not achieved, the possibility is left open that in some cases a pure catabolic or antianabolic response to trauma may occur. It is equally possible, however, that in such cases insufficient nitrogen was given. It is also pointed out that the attainment of nitrogen balance does not mean that individual protein functions may not still be disturbed. It may be that some tissues build protein while others do not and such a derangement may not be reflected by the over-all nitrogen balance.

EFFECTS OF ANTAGONISTS TO PTEROYLGLUTAMIC ACID

Antagonists have been developed for a relatively large number of metabolites (R. O. Roblin, *Chem. Rev.* **38,** *255 (1946)*). With the synthesis and development of pteroylglutamic acid (PGA), studies are being reported of compounds antagonistic to this vitamin. G. J. Martin, L. Tolman, and J. Moss (*Arch. Biochem.* **12,** *318 (1947)*) briefly reported the synthesis of a compound termed d(-)methylfolic acid which was described as a "displacing agent" for pteroylglutamic acid. This compound was prepared by the method of R. B. Angier and co-workers (*Science* **103,** *667 (1946)*) for PGA, except that 2,3-dibromobutyraldehyde replaced 2,3-dibromopropionaldehyde and the unnatural glutamic acid moiety replaced the natural (l) glutamic acid compound used in the original synthesis. d(-)Methylfolic acid was shown to be an effective displacing agent for PGA in assays using Streptococcus faecalis R as the test organism. The effective ratio of inhibitor to metabolite was approximately 150.

In man, the inactivity of this compound as a substitute for PGA was reported briefly by T. D. Spies, R. E. Stone, and R. O. Brandenburg (*South. Med. J.* **40,** *618 (1947)*). A patient with sprue in relapse failed to respond to 20 mg. of methylfolic acid by mouth daily for a twelve day period. He subsequently responded to doses of 10 mg. of PGA orally. Another patient, who had pernicious anemia in relapse and who had responded previously to administration of PGA, failed to respond to 40 mg. of methylfolic acid by mouth daily for a ten day period. The Birmingham investigators concluded that methylfolic acid was ineffective in stimulating blood regeneration. Their report does not indicate whether the administration of methylfolic acid to these subjects brought about an exacerbation of their disease, as might be expected if the compound exhibited a displacing effect in man.

An antagonist which presumably differs from that prepared by Martin and coworkers only in that the glutamic acid moiety is the natural (l) form has been prepared by M. E. Hultquist and J. M. Smith, and its effect on rats investigated by A. L. Franklin, E. L. R. Stokstad, M. Belt, and T. H. Jukes (*J. Biol. Chem.* **169,** *427 (1947)*).

The crude compound was not purified and was employed directly in the experiments to be discussed. This antagonist was demonstrated to inhibit the growth-promoting effect of PGA on both Lactobacillus casei and S. faecalis R. The inhibition was reversible by PGA. The minimum ratio of antagonist to metabolite was reported as 20 to 30 for S. faecalis R and 1000 for L. casei.

These workers then investigated the effect of the crude antagonist on rats. Female albino rats were fed a basal diet containing glucose, Labco washed casein, salt mixture, and corn oil, plus the usual fat and water soluble vitamins except pteroylglutamates. In addition, the diet contained 1 g. per 100 g. of succinylsulfathiazole. Sixteen groups of 5 rats each were placed on the basal diet supplemented with varying amounts of PGA and/or the antagonist. When the level of pteroylglutamic acid was 0.3 mg. or less per gram of antagonist, the animals developed acute signs of PGA deficiency which became more intense as the ratio of antagonist to metabolite was increased. The syndrome was characterized by a loss in weight, inanition, the development of a rough unkempt fur, encrustation of a red pigment on the vibrissae, a severe diarrhea, and hematologic changes including a reduction in hemoglobin concentration, a pronounced reduction of the white cell count, and a relative granulocytopenia. Addition of the antagonist alone to the basal diet shortened the time required for the development of the syndrome. The hematologic effects may be illustrated by the following data on the fifth week of the experiment for hemoglobin (grams per 100 ml.), white cell count (thousands per cubic millimeter), and granulocyte count (cells per cubic millimeter), respectively: basal diet alone, 18.3, 12.6, 1500; basal diet plus 1 mg. per kilogram PGA, 19.3, 15.2, 2600; basal diet plus 1 g. per kilogram of antagonist, 10.5, 0.8, 16; basal diet plus 3 mg. per kilogram PGA and 1 g. per kilogram antagonist, 20.9, 13.7, 1100. The cytologic findings in the bone marrow were in accord with the hematologic changes observed in the peripheral blood. The animals developing the syndrome usually became moribund within one to two weeks after the onset. At autopsy the most significant findings were necrotic and ulcerative changes in the oral cavity. The gastrointestinal tract in most animals was found to be empty and atonic. The resemblance of these findings to those in the vitamin M deficient monkey (W. C. Langston, W. J. Darby, C. F. Shukers, and P. L. Day, *J. Exp. Med.* **68,** *923 (1938)*) is obvious.

After one group of 5 animals developed the syndrome, the supplementation of PGA was increased to 100 mg. per kilogram of diet. One animal died the following day, but the remaining 4 showed rapid clinical and hematologic responses. This syndrome in rats, therefore, could be reversed as well as prevented by PGA.

These workers note that several clinical syndromes are characterized by increased numbers in the cellular elements of the blood. They suggest that this antagonist might be used in experimental attempts to modify the course of such blood dyscrasias. One can be certain that such therapeutic studies will be made in the near future and their results will be awaited by both experimentalists and clinicians.

These same workers (A. L. Franklin, E. L. R. Stokstad, and T. H. Jukes, *Proc. Soc. Exp. Biol. Med.* **65,** *368 (1947)*) in a similar experiment, employing female mice 5 to 6 weeks old, found that signs of PGA deficiency did not develop within a period of six weeks on the diet alone, but when the crude antagonist was added a syndrome appeared which was characterized by depression of growth, anemia, and leukopenia. These changes could be prevented by the addition of PGA to the diet. Day-old New Hampshire chicks placed on a purified diet free of PGA developed the usual signs of deficiency including slow growth, poor feathering, and low hemoglobin content of

the blood. The development of this deficiency syndrome in chicks was aggravated by the addition of the antagonist to the diet and again the effects of the antagonist were reversible by the vitamin.

Finally, a report has appeared of the effect of this antagonist on one pig (A. D. Welch and co-workers, *Proc. Soc. Exp. Biol. Med.* **65,** *364 (1947)*). Five Berkshire pigs were maintained on a purified diet of Labco casein, glucose, hydrogenated vegetable oils, corn oil, salt mixture, and the usual fat soluble and water soluble vitamins including biotin but excluding PGA. Succinylsulfathiazole was added to the diet. Two of the pigs were given daily oral supplements of PGA, 0.02 and 0.2 mg. per kilogram, respectively. Three of the pigs were fed the basal diet alone and after sixteen days on the diet one of these animals was given a supplement of the crude antagonist. No disturbances in growth or hematopoiesis were seen in the 2 pigs receiving the purified diet containing succinylsulfathiazole. This observation is in contrast to the report based on one experimental pig in which an anemia developed under a similar regimen (*Nutrition Reviews* **4,** *250 (1946)*). By contrast, the pig given the crude antagonist of PGA showed a decreased rate of growth (there was no loss of weight, however), developed a patchy alopecia, and by the eightieth day an anemia had developed. At this time the vitamin-free casein of the diet was replaced by crude sodium caseinate and the pig was given by gastric intubation an extract of crude casein equivalent to 100 g. of casein together with 80 to 150 ml. of fresh human gastric juice. The authors state "None of the materials administered contained appreciable amounts of microbiologically determinable PGA." Within ten days clinical improvement was evident and a reticulocytosis appeared. This was followed by a remission of the anemia. The data presented, however, do not indicate that the white cell count returned to pre-experimental levels.

It is difficult indeed to interpret the significance of this report. Particularly is this true since the pig continued clinically improved for an additional seventy days despite cessation of the therapy described and return to the original purified succinylsulfathiazole-containing diet and supplements of the antagonist. Until additional experiments are presented it would appear that the authors' implication that their study affords a "suitable bioassay" for antianemic substances is premature. Furthermore, additional evidences must appear which indicate a fundamental relationship between this syndrome and pernicious anemia before it is permissible to reason that findings bearing on one of these conditions are directly applicable to the other.

PHENYLALANINE AND TYROSINE CONTENTS OF CHICKS AND EGGS

C. R. Grau (*J. Biol. Chem.* **168,** *485 (1947)*) points out that one of the most direct methods of studying the metabolism of closely related amino acids is to estimate the amino acid contents of the animal carcass at the start of the experiment, to calculate the intake from feed consumption data, and to determine the amino acids present at the end of the experiment. By the use of suitable diets, information can thus be obtained concerning the extent of conversion of one amino acid into another and the efficiency of utilization of amino acids under various feeding regimens. The report made in this paper deals with the determination of the phenylalanine, tyrosine, and nitrogen contents of chick carcasses at various stages of growth and with certain changes which occur as egg protein is transformed into chick protein.

After surveying methods which have been used for the determination of the phenyl-

alanine and tyrosine of tissue proteins, the author developed modifications of existing methods. For phenylalanine the method of R. Kapeller-Adler (*Biochem. Z.* **252,** *185* (*1932*)) as modified by A. A. Albanese (*J. Biol. Chem.* **155,** *291* (*1944*) was used. This method involves nitration of the phenylalanine and measurement of the final product in an alkaline solution at 500 millimicrons. Evidence was presented that at this wavelength the absorption due to histidine, tyrosine, and tryptophane was very small, so that the presence of these amino acids would not cause any serious error and consequently there is no need to attempt to remove them from the solutions. The color varied with concentration of phenylalanine in the manner predicted by Beer's law.

For the determination of tyrosine, the Millon reaction was employed essentially as developed by O. Folin and A. D. Marenzi (*J. Biol. Chem.* **83,** *103* (*1929*)) and modified by R. J. Block and D. Bolling ("The Amino Acid Composition of Proteins and Foods," *Thomas, Springfield* (*1945*)). No interference by phenylalanine, histidine, or tryptophane with the reaction occurred.

Chicks were analyzed after killing with carbon tetrachloride. The intestinal tract was opened, washed, and put with the rest of the carcass into a widemouthed conical flask for digestion. The weight of the carcass thus obtained was termed the "empty weight." After eighteen to twenty-four hours the digest was diluted with hot water and filtered and further diluted so that 5 ml. aliquots could be used for phenylalanine analysis and 3 ml. aliquots for tyrosine determinations, while micro-Kjeldahl analysis of the filtrate was made for nitrogen. Chicks of several ages were analyzed for phenylalanine, tyrosine, and nitrogen by these methods, groups of 4 being taken every five days for thirty days. From one group of newly hatched chicks the yolk sacs and membranes were dissected out for separate analysis.

All the chicks were fed a commercial type chick mash from hatching until they were killed; they were not fasted before death. The empty weight ranged from 77 to 91 per cent of the live weight, with a mean value of 87 per cent. The average phenylalanine and tyrosine contents of chick carcass protein (calculated to 16 per cent nitrogen) were 5.61 and 3.26 per cent respectively. The value for tyrosine lies within the range reported by Block and Bolling (*loc. cit.*) for rats, but that for phenylalanine is somewhat higher than the value for rat carcass protein.

Four eggs including shell and membranes were hydrolyzed in the same manner as the chicks and analyzed. The average value for phenylalanine was somewhat higher than that recorded by B. Munks, A. Robinson, E. F. Beach, and H. H. Williams (*Poultry Sci.* **24,** *459* (*1945*)) for similar sized eggs, while the tyrosine values were closely similar. The efficiency of the embryo in using the nutrients available to it may be expressed as a percentage recovery (carcass content $\times$ 100 $\div$ egg content), but probably a more adequate measure of efficiency is obtained when the yolk sac contents of the hatched chick are taken into account. About 15 per cent by weight of the newly hatched chick is yolk material (S. L. Parker, *Hilgardia* **4,** *1* (*1929*)) which has not been metabolized and so should not be included in the efficiency calculations.

Leaving out the yolk sac protein, the average figures obtained for the efficiency calculations showed that 90 per cent of the egg protein was converted into chick protein, while about two thirds of each of the amino acids, phenylalanine and tyrosine, present in the egg were transferred to chick tissue. These high efficiencies of utilization of egg amino acids are not approached by the chick after hatching; unpublished data of the author suggest that a 40 per cent recovery of feed amino acids in the carcass is fairly high.

The detailed figures given by the author

suggest that there is no interchange of phenylalanine and tyrosine during embryonic development, an important fact established by this study.

DISEASE SYNDROMES RELATED TO VITAMIN B-COMPLEX DEFICIENCIES

Within the last few years a number of disease syndromes have been identified as deficiencies of the vitamin B-complex. Some of these have not been too clearly defined. Especially ill defined are those syndromes predominated by neurologic signs. An earlier review discussed the optic neuropathy which has been associated with severe malnutrition (*Nutrition Reviews* **5,** *312 (1947)*). These neuropathies frequently became manifest in the absence of the signs of classic deficiency diseases such as pellagra and beriberi, and the causative deficiency was not defined.

During the Spanish Civil War, 1936 to 1939, M. Peraita (*Brit. Med. J.* **2,** *784 (1946)*) studied a large group of patients suffering from deficiency diseases. From these he selected 98 cases with pronounced nervous disorders, but with no skin symptoms suggesting pellagra, and which exhibited no true beriberi. The most frequent symptoms were paresthesias which occurred predominately in the toes. In fact, this writer refers to the neuropathy as the "painful feet" syndrome. It was characterized by a pricking or stinging to stab-like pain in the feet or sometimes in the hands. The patients often complained that their feet were numb and cold during the day but very hot at night. Pronounced hyperesthesia of the feet was frequent. Among other symptoms occurring in this series of cases were depression, lacrimation, insomnia, forgetfulness, reduced acuity of special senses, loss of hair and nails, and changes in the motor functions of the gastrointestinal tract. Peripheral motor defects and cardiac and skin lesions were rare, but cutaneous and proprioceptive sensory changes were frequent. Neither thiamine nor niacin was curative, but the lesions responded to oral administration of 90 g. of dry yeast daily. The effective agent in the yeast was not identified.

Another neurologic disorder which appears to be a deficiency state has been reported by P. R. Graves (*Brit. Med. J.* **1,** *253 (1947)*). Graves refers to this syndrome as pellagrous encephalopathy. He states that the syndrome occurred as a small outbreak following an epidemic of thiamine deficiency and later epidemics of B-complex deficiencies. The thiamine deficiency was manifest by neuritic and wet beriberi and Wernicke's encephalopathy. In contrast to the predominantly sensory changes encountered in the "painful feet" syndrome described above, this neurologic disorder was remarkable because of the appearance of spastic paraplegia, or paralysis of the legs and lower part of the body. The onset varied in severity from mild extensor plantar responses to a state of deep coma. The residual symptoms ranged from none to a state of persistent spastic paraplegia.

From a study of 63 cases of this deficiency syndrome which were encountered among prisoners of war in Singapore, Graves described the clinical picture as one initiated by various combinations of locomotor disability (weakness, stiffness, or unsteadiness of the legs), failure of vision, diplopia, pains in the feet or back, paresthesias in the feet, cramps, mental depression, nocturnal incontinence of the urine, and, in 2 cases, epileptiform seizures. All cases exhibited marked spasticity with or without extensor plantar dysfunctions. The mental changes were described as general dawdling, depression, loss of emotional control, and hysterical behavior.

Six fatal cases were observed in this series. Attempts at therapy included the addition

to the diet of reconstituted milk, Marmite, nicotinamide, thiamine, liver extract, and red palm oil in addition to supplements of rice polishings, peanuts, eggs, canned meat, and fresh vegetables as available. The course of the disease was not altered appreciably by any of these additions.

Although Graves refers to this syndrome as pellagrous encephalopathy, he notes that not more than one third of the cases which he reported exhibited other evidences of deficiencies. The quantity of nicotinamide administered to these patients is not reported, but the length of treatment was "for a maximum of eleven days." It does not appear that the identity of this syndrome with the lesions of the nervous system observed in pellagra in the United States has been established. These manifestations of pellagra were noted by D. T. Smith, J. M. Ruffin, and S. G. Smith (*J. Am. Med. Assn.* **109,** *2054* (*1937*)) and by J. M. Ruffin and D. T. Smith (*South. Med. J.* **32,** *40* (*1939*)) to respond within a period of a week to amounts of niacin varying from 30 to 60 mg. per day. Furthermore, the universal occurrence of spastic paraplegia was not characteristic of pellagra during the epidemics in the southern United States.

The many reports of clinical neurologic syndromes occurring among malnourished individuals indicate that there exists a fertile field of investigation in the effects of dietary deficiencies on the nervous system. In most cases to date the actual causative deficiency remains unidentified.

PRODUCTION OF METHIONINE *IN VITRO* BY TRANSMETHYLATION

H. Borsook and J. W. Dubnoff (*J. Biol. Chem.* **160,** *635* (*1945*)) reported the methylation of guanidoacetic acid to creatine by rat liver slices in the presence of choline together with either homocysteine or homocystine, while choline, homocysteine, or homocystine alone had little effect. The same authors established earlier (*Ibid.* **132,** *559* (*1940*)) that liver slices of the cat, rabbit, and rat convert guanidoacetic acid into creatine, and that this methylation is accelerated in rat liver by methionine, which was the only substance found among a large number of amino acids, methylated amines, and other compounds which were able to effect this methylation in rat liver. The first-mentioned observations served to link this evidence, obtained from tissue-slice experiments with the transmethylation studies of V. du Vigneaud *et al.* (V. du Vigneaud, J. P. Chandler, A. W. Moyer, and D. M. Keppel, *Ibid.* **131,** *57* (*1939*); V. du Vigneaud, J. P. Chandler, M. Cohn, and G. B. Brown, *Ibid.* **134,** *787* (*1940*)) performed *in vivo*. H. Borsook and J. W. Dubnoff (*Ibid.* **169,** *247* (*1947*)) now report the results of a study of methionine formation by transmethylation in rat kidney and liver and kidney slices and homogenates, with especial attention to inhibitors of the reaction, both aerobic and anaerobic.

The results of the experiments reported have definitely established that there are at least two different categories of transmethylation reactions. The methylation of guanidoacetic acid to creatine is an example of the first type, which is dependent on oxygen and is inhibited by oxidation inhibitors such as cyanide, oxide, arsenite, and arsenate. The methylation of homocysteine or homocystine to methionine is an example of the second type, with choline or betaine as the methyl donor, which does not require oxygen and is not inhibited by oxidation inhibitors. Methionine itself can also, of course, act as methyl donor, behaving as a first type methyl donor, whereas choline or betaine are unable to do so, unless homocysteine or homocystine is present to act as a "methyl carrier." Liver homogenates were unable, with or without added methionine, to methylate guanidoacetic acid to

creatine, although they retained their ability to catalyze the formation of methionine by transmethylation.

In type 1 reactions the direction of the transfer is from an S-methyl to an N-methyl group, while in type 2 reactions it is just the reverse; although it must be borne in mind that this distinction may not be fundamental, and the rule may not hold when more transmethylations become known.

A methylation reaction by means of which a number of mammals partly dispose of an excess of ingested niacin, was demonstrated for rat liver by W. A. Perlzweig, M. L. C. Bernheim, and F. Bernheim (*J. Biol. Chem.* **150,** *401* (*1943*)). They showed that this reaction is strictly aerobic, that it is carried out by whole cells but not by homogenates, and that it is usually (but not always) accelerated by the addition of methionine. This reaction is thus of a type similar to the methylation of guanidoacetic acid.

Choline and betaine were the only substances observed to act additively as methyl donors in the presence of those donors already present in the liver slices. The interrelation between these two compounds is not yet fully understood. DeW. Stetten (*J. Biol. Chem.* **138,** *437* (*1941*); **140,** *143* (*1941*)) has suggested on the basis of studies with various compounds labeled with N^{15} that betaine could be demethylated to glycine, which could then be reduced to ethanolamine and methylated by methionine to choline. In the above N^{15} study the fate of the methyl groups of betaine was not directly ascertained; the present observations of Borsook and Dubnoff indicate, however, that a methyl group of betaine would be available for choline formation by way of its incorporation into methionine, without excluding other routes of transfer.

As a final point made by the authors, it is emphasized that although betaine causes more rapid methyl transfer than choline, it is unlikely that the oxidation of choline to betaine is obligatory for the purpose. If it were, little methionine formation would be expected from choline under anaerobic conditions, whereas choline has been shown to be active under such conditions.

ENVIRONMENTAL TEMPERATURE, THYROXINE, AND FOOD SELECTION IN MICE

Data are available which demonstrate rather convincingly that animals given free choice of foods will select and adapt their food intake with considerable accuracy according to their needs. T. B. Osborne and L. B. Mendel (*J. Biol. Chem.* **35,** *19* (*1918*)) early found that rats and mice confronted with two experimental diets would select the more complete one; similarly, following a period of protein starvation, the consumption of protein rich food is increased (E. M. P. Widmark, *Acta physiol. Scandinav.* **7,** *147, 278* (*1944*)). Adrenalectomized and parathyroidectomized animals permitted free selection increase their intake of sodium chloride and of calcium, respectively, a response which tends in each case to minimize the effects of their physiologic inadequacies (C. P. Richter, *Am. J. Physiol.* **115,** *155* (*1936*); C. P. Richter, L. E. Holt, and B. Barelare, *Ibid.* **122,** *734* (*1938*)).

S. Donhoffer and J. Vonotzky (*Am. J. Physiol.* **150,** *329* (*1947*)), in experiments conducted in 1942 but published from Hungary only this year, have extended these experiments to determine the effect of environmental temperature upon selection of foodstuffs. Two control and two experimental groups each containing 10 mice were used for each experiment. The animals were allowed the choice of three experimental diets. Each of these diets contained one-third part of a standard mixture and two-thirds parts of starch, casein, or

lard. The standard mixture contained salts, dried brewers' yeast, casein, and ground maize, and was supplemented in each mixture by 1 to 3 per cent of whole milk. Each of the three diets given alone, provided adequate nourishment and maintained health over a period of at least three months. Water was provided ad libitum and the intake of each mixture was measured daily.

After a preliminary period of two to three weeks at room temperature, the experimental groups were placed in an environment controlled thermostatically from 29 to 33°C., or in a refrigerator where the temperature was 10 to 11°C. Following expectations, food intake increased at the low temperature sufficiently to provide 20 to 40 per cent more calories than were ingested in the warm environment. The change was due almost entirely to increased intake of the high carbohydrate mixture at the low temperature and decreased intake of the same mixture at the higher temperature. Consumption of the "high protein" and "high fat" mixtures throughout the whole experiment remained practically unchanged. The same changes in food intake were observed serially when a group of animals was maintained first in the warm environment, followed by a period in the cold environment, and finally a return to the warm environment. That mice exposed to low temperature selected the necessary additional calories to maintain weight by eating more starchy food, exclusively, appeared the more significant to the authors because at room temperature they preferred fatty food, which ordinarily furnished about two thirds of the total calories ingested.

In attempting to explain the observed selective increases in carbohydrate consumption, the authors cite observations of various investigators which demonstrate a thermoregulatory function of the thyroid and seasonal changes in thyroid activity, and suggest that changes in thyroid function may play a role in qualitative regulation of food intake. To test this idea, free selection experiments similar to those described earlier were conducted (S. Donhoffer and J. Vonotzky, *Am. J. Physiol.* **150,** *334 (1947)*) in which the food intake of normal mice was compared with that of mice receiving 0.02 to 0.04 mg. of thyroxine daily by subcutaneous injection. Thyroxine administration resulted in the usual rise of 50 to 100 per cent in oxygen consumption, followed after a lag of three to six days by increased food intake. In all experimental groups the consumption of starchy food remained practically unchanged or rose slightly, and consumption of the high protein diet remained unchanged. In some experiments the intake of all three foods increased at the start of thyroxine treatment, but later protein and fat consumption decreased to or below the original level and an elective increase in the consumption of starchy food was observed. At the height of the thyroxine effect, more than 50 per cent (sometimes as high as 80 per cent) of the total calories ingested were derived from the carbohydrate-rich food, whereas before and after thyroxine treatment the normal preference of the animals resulted in 50 to 80 per cent of the calories being consumed as the fat-rich diet. Thus prolonged administration of thyroxine is associated with changes in food selection essentially similar to, though much more pronounced, than those observed in animals exposed to low temperatures. The observed rise in carbohydrate consumption at low temperatures may thus be due to changes in thyroid function. Alternatively, it is possible that the shift in food preference results from some unknown factor associated with the increased metabolic rate common to animals maintained at low temperatures and to those receiving thyroxine supplementation.

NOTES

Letters to the Editor

Dear Sir:

The review entitled "Folic Acid and Niacin Deficiency in the Dog" (*Nutrition Reviews* **5,** *205 (1947)*) may leave the impression that folic acid deficiency alone underlies the anemia which develops in dogs ingesting a Goldberger-type diet. While it seems possible that folic acid deficiency is responsible for the moderate anemia which can be elicited by subjecting dogs to repeated bouts of "blacktongue," and that the common anemia of alcoholic pellagrins is probably due to a mixed niacin-folic acid deficiency, it should not be overlooked that niacin itself has a hematopoietic role. P. Handler and W. P. Featherston (*J. Biol. Chem.* **151,** *395 (1943)*) have demonstrated this in dogs with profound niacin deficiency tided over blacktongue crises by parenteral saline therapy, and in adult dogs on a synthetic niacin deficient ration without prominent signs of blacktongue. Such animals may have a total circulating hemoglobin of as low as 10 per cent of normal, and upon administration of niacin show a prompt reticulocytosis (levels up to 35 per cent) and return to normal hemoglobin levels within two months.

From the biology of the erythrocyte it is obvious that hematopoiesis must demand an adequate supply of all essential nutrients and that, under proper conditions, anemia must result in all deficiency states. Demonstration of this anemia will depend on whether or not the quantitative requirements of the various tissues for a given nutrilite are such that death from other causes may intervene before the anemia is established. With the possible exception of mineral elements, there is no evidence indicating the existence of any nutrilite which serves solely as a hematopoietic factor. Thus, while the relationship between folic acid and the "maturation factor" is not clear, it is evident from its ubiquitous occurrence and the fact that it is required even by microorganisms, that this material is a significant metabolite in virtually all cells and it is only a combination of quantitative circumstances which leads to anemia as the striking manifestation of this deficiency.

Philip Handler
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of Medicine
Durham, North Carolina

Dear Sir:

In your issue for June of last year (*Nutrition Reviews* **5,** *164 (1947)*), summarizing recent work on the use of vitamin E in heart disease, you remark in passing that "early attempts to demonstrate a role for vitamin E in preventing abortions in women failed." It is now some years since I made an examination of the data then available on the treatment of habitual abortion in women by administration of wheat germ, wheat germ oil, or vitamin E concentrates (*Brit. Med. J.* **1,** *890 (1940)*).

My examination had partly the immediate object of testing the point at issue, but it was also designed to call attention to a method of assessing clinical trials too infrequently applied, in my view. Setting up a series of randomized alternate cases, treated with a drug and receiving a placebo, is difficult, if not impossible, when very large numbers are not available at the same time and in the same place. Yet previous records from hospitals and institutions might be expected frequently to provide base line data for the number of spontaneous abortions occurring without treatment or under treatment known not to be effective. It was the publication by P. Malpas (*J. Obstet. Gynec. Brit. Empire* **45,** *932 (1938)*) that provided an opportunity to compare, by

the use of simple statistical methods, the results of vitamin E therapy with what might have been expected in its absence. It was, of course, necessary to make certain assumptions, all of which, I believe, were clearly stated in my paper, which led to the conclusion that the chances of the results recorded being due to sampling error were statistically negligible; in other words, the treatment would seem to have been effective in a high proportion of cases. My assumptions may have been wrong, but my conclusions have never been challenged, although statements such as those made in your article continue to appear in journals on both sides of the Atlantic. Either my conclusions were correct, in which event these statements are wrong, or my conclusions were incorrect, in which event one would expect them to have been challenged and disproved.

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Food Habits and Tooth Decay

H. H. Neumann (*J. Roy. Inst. Pub. Health Hyg.* **9**, *373 (1946)*; *Lancet* **I**, *806 (1947)*) has recorded some philosophic observations on the distribution of dental caries in various nations of the world and has presented some suggestions as to the causes of tooth decay. This writer notes that the cause of tooth decay has been attributed by various investigators to widely different factors such as heredity, hormonal disturbances, racial susceptibility, deficiencies of certain elements in the local soil and water and consequently in the dietary, over-refined food, vitamin deficiencies, and excessive sugar and starch consumption. Neumann discussed each of the above hypotheses in terms of the dental caries incidence of selected nations and concluded that the environment and food habits of these nations did not permit the acceptance of any one of these hypotheses. In a further examination of diets used in these countries, he postulated that the primary etiologic factor of dental caries was a deficiency of tough food requiring mastication. This deficiency was not believed to result in too little detergent action of the food during eating, but to cause a disuse atrophy provoking a decalcification of the dental structures. The term "disuse odontoporosis" was coined for this postulated condition with the suggestion that it might be comparable to the osteoporosis observed in bones after a period of disuse. Neumann suggested that the prevention of dental caries could be achieved ideally by the addition of soft sugar cane to the diet or more practicably through the consumption of some tough breadcrusts daily. Obviously the above hypothesis of the cause and prevention of dental caries is based entirely on presumptive reasoning. This postulate seems especially extended in view of the fact that decalcification of formed teeth has never been demonstrated under the most extreme metabolic conditions. Yet no one can deny that the use of cutlery and modern refining and cooking procedures have to a large extent relieved the teeth of civilized man from the tasks of biting and chewing for which they are admirably constructed.

Recent Nutrition Books

The Bacterial Cell. Rene J. Dubos. Harvard University Press, Cambridge, Mass. 1947. Pp. 460. (Third Printing)

The Dynamic State of Body Constituents. Rudolf Schoenheimer. Harvard University Press, Cambridge, Mass. Pp. 78. (Reprinting)

Calcium and Phosphorus in Food and Nutrition. Henry C. Sherman. Columbia University Press, New York. 1947. Pp. 176.

Nutrition and the Public Health. A Study Guide. Adelia M. Beeuwkes and John J. Hanlon. The Overbeck Co., Ann Arbor, Michigan. 1947. Pp. 120.

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